

ological significance of the Ephs and ephrins in insulin secretion *in vivo* by creating and characterizing β cell-specific inducible knockouts of specific Ephs and/or ephrins. It is notable that EphA5 is localized to the insulin secretory granule, an organelle that is also associated with ATP-dependent K channels, glucokinase, and the insulin receptor (Geng et al., 2003; Hribal et al., 2003). Whether the close association between these proteins is necessary for intracrine regulation at the level of the secretory granule is worth investigating.

Glucose-stimulated insulin release typically occurs in two phases—a rapid first phase (2–3 min in the mouse and 10 min in humans) after glucose stimulation and a prolonged second phase that lasts up to 30 min. In contrast to many factors—such as the insulin receptor tyrosine kinase—that regulates the first phase glucose-induced insulin release (Kulkarni et al., 1999),

Eph-ephrin signaling predominantly affects the second phase of insulin release. Another avenue to pursue is whether there might be interaction between these two RTK systems that both regulate β cell function and insulin secretion.

Although a part of the mystery underlying β cell-to- β cell communication has been unraveled, the relevance of these observations to disease needs clarification. Could abnormalities in the expression of Ephs and ephrins contribute to defects in altered insulin secretion or maintenance of β cell mass that are characteristic of type 2 diabetes or other insulin-resistant states? Are there polymorphisms in the genes that code for Ephs and ephrins that are associated with altered glucose homeostasis in humans? Further integrative studies should help define the functions of Ephs and ephrins and their role in diabetes and metabolic diseases.

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Targeting the Sticky Fingers of HIV-1

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Although human blood plasma contains molecules that inhibit the activity of HIV-1, their identity is largely unknown. Münch et al. (2007) now identify a peptide corresponding to a portion of α 1-antitrypsin that potently inhibits entry of HIV-1 into host cells by binding to a hydrophobic segment of the viral envelope glycoprotein gp41.

A number of molecules have been discovered that can inhibit the entry of HIV-1 and other viruses into cells of their host (reviewed in Dimitrov, 2004; Liu et al., 2007). Perhaps the most well-known are the anti-HIV peptides SJ-2176 (Jiang et al., 1993) and T20 (Wild et al., 1994) derived from the C-terminal heptad repeat region of the viral envelope glycoprotein gp41. T20 remains the

only HIV-1 entry inhibitor approved for clinical use by the US Food and Drug Administration. The search for peptides with similar or greater potency and crossreactivity and improved pharmacokinetics has yet to yield additional peptide-based therapeutics of clinical utility. Following the example of T20, research has remained largely focused on peptides derived from viruses. How-

ever, human blood plasma contains naturally occurring low-molecular-weight protein inhibitors of HIV-1 infection. Although the possibility of finding inhibitory peptides in human blood plasma has long been recognized, until recently the task of identifying them from a mixture of more than one million different peptides and small proteins has been daunting. Now, using a peptide library

extracted from 10,000 liters of human hemofiltrate, Münch et al. (2007) have identified a peptide—VIRIP for VIRUS-INHIBITORY PEPTIDE—that potently inhibits HIV-1 entry into cells.

Münch et al. used a straightforward, systematic, and labor-intensive approach to identify VIRIP hidden among an enormous number of other peptide molecules. They fractionated peptides from hemofiltrate by cation-exchange and reverse-phase chromatography into 322 fractions and identified one that inhibited HIV-1 activity without cellular toxicity. Further analysis of this fraction revealed VIRIP, a 20-residue peptide corresponding to the C-proximal region of the most abundant circulating serine protease inhibitor α 1-antitrypsin. VIRIP inhibited primary isolates of HIV-1 from different clades and inhibited HIV isolates that use different coreceptors for entry. VIRIP also inhibited variants of HIV resistant to protease and reverse transcriptase inhibitors as well variants resistant to the fusion inhibitor T20.

The inhibitory activity of VIRIP was further improved by a systematic analysis of the relationship between structure and activity. Residues critical for its activity were initially identified, and then a series of more than 600 chemically synthesized VIRIP analogs were analyzed. Several of these VIRIP derivatives containing intramolecular disulfide bonds and other modifications exhibited neutralizing activity toward HIV in vitro up to two orders of magnitude higher than that of VIRIP. Although the neutralizing activity of these VIRIP derivatives is comparable to that of T20, T20 inhibits cell fusion at significantly lower concentrations than these peptides. What causes these differences is not clear and may depend on the assay used and the isolate tested. Cell-to-cell transmission of HIV-1 is much more efficient than transmission by cell-free virus (Dimitrov et al., 1993) and could be dominant in vivo. In contrast to other inhibitors including T20, HIV-1 was not able to generate resistant strains to one of the most extensively characterized VIRIP variants (VIR-576). If replicated

in humans this property alone of VIR-576 would set it apart from existing inhibitors that affect other stages of the HIV-1 life cycle.

Remarkably, the target for VIRIP turned out to be the fusion peptide region of HIV-1 gp41, a glycoprotein that forms a trimer with gp120. The identification of the hydrophobic fusion peptide as the target is surprising because its role in the fusion cascade leading to virus entry has been elusive (Gallo et al., 2003). Moreover, raising antibodies against this region has proven exceedingly difficult (C.C. Broder, personal communication). According to the “sticky finger” model, drawn in many cartoons of the HIV-1 fusion reaction, the fusion peptide inserts into the target cell following the change of conformation of gp120-gp41, called “triggering,” that occurs subsequent to binding of gp120 to host cell CD4 and coreceptors. Yet, if the fusion peptide is a target for VIRIP, which binds to the aqueous form of the fusion peptide, this poses a conundrum. This is due to the fact that gp41 may rapidly dip its sticky finger into the bilayer once the trigger has been pulled. Consequently, the sticky finger should avoid interference by VIRIP because the bilayer-inserted fusion peptide will no longer be available for binding to VIRIP. Indeed, attempts have been made to inhibit the HIV-1 gp41 fusion process after insertion of the fusion peptide into the membrane by designing membrane-anchored fusion peptide analogs (Gerber et al., 2004). Exposing the sticky finger prior to triggering would have deleterious consequences for gp120-gp41 (such as aggregation). Because we do not have a high-resolution structure of the gp120-gp41 trimer we can only speculate about the disposition of the fusion peptide in the overall structure. One speculation is that after the gp120 interaction with CD4 and coreceptors the fusion peptide spends some time between the viral and cell membranes disrupting the water structure and the respective repulsive hydration forces, and this leads to spontaneous local fusion (Dimitrov, 1996). Further studies addressing the

timing and location of VIRIP action may not only uncover new features of how it inhibits HIV entry but may also shed light on the elusive role of the fusion peptide itself.

Among the many implications of this study perhaps the most important and urgent question, especially for individuals carrying HIV-1 isolates resistant to other drugs, is whether VIRIP variants, such as VIR-576, have therapeutic potential. Although only clinical trials could definitely answer this question, one could speculate that such a possibility exists, with T20 providing an example of success. The unique mechanism of inhibition by VIRIP combined with a hopefully low level of resistant mutants holds promise for the use of this inhibitor in combination with other drugs. VIRIP and its variants are also the first HIV-1 fusion inhibitors identified by the systematic screening of naturally occurring mixtures of very high complexity. Even if the discovery of VIRIP does not lead to a new HIV-1 therapeutic, the methodology will undoubtedly have a profound impact on future research. Although labor intensive, the ability to screen human blood plasma and select peptides with predefined properties promises to yield new compounds of importance for other diseases. Finally, these results suggest yet another possible mechanism of resistance to HIV infection—the induction of large amounts of inhibitory peptides during inflammation. How significant this mechanism might be in delaying the progression of HIV disease is an intriguing question that remains to be answered.

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Molecules to Remember

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“Working memory” is used for the transient storage of information in the brain. In this issue of *Cell*, Wang et al. (2007) now reveal how a series of molecular events involving α 2A-adrenoceptors and a class of ion channels gated by cAMP tune the responses of neural circuits that function in working memory in mammals.

For short periods of time, the human brain can consciously hold onto thoughts. This cognitive function, which is referred to as working memory, allows us to respond efficiently to the varying environmental demands of everyday life (Baddeley, 1986). Consider, for example, a waiter who takes your order at a restaurant without using a notepad. To relay your order to the cook, he must maintain in working memory all the dishes you wanted plus any special requests. This information is short-lived—it may be erased once the food is brought to your table—but in the meantime it should be resistant to distractions, such as conversations with other people on the way to the kitchen. Although this is a complex cognitive function, a large body of experimental work has shown that neural circuits in the brain's prefrontal cortex mediate working memory.

In this issue of *Cell*, Wang et al. (2007) report in several mammalian models the results of an experimental tour de force aimed at understanding some of the key molecular processes that regulate neural circuits

of the prefrontal cortex during tasks that require spatial working memory. They discovered that the mental representation of a spatial location held in working memory depends on the interaction between the postsynaptic α 2A-adrenoceptors (α 2A-ARs) and a class of ion channels called hyperpolarization-activated cyclic nucleotide channels (HCNs), which are gated by cAMP. This is a remarkable advance, not only because they tease out the functional interaction between these molecules and their effect on working memory, but also because the results provide a rational basis for the development of clinical therapies to treat mental disorders involving the prefrontal cortex.

Funahashi et al. (1989) originally showed that, in monkeys performing a task involving spatial working memory, neurons in the prefrontal cortex respond selectively to memorized locations. In this now classic task, a small visual cue is briefly presented along a circle, and monkeys have to remember its position for few seconds during what is called the delay period; the position of the

cue changes from trial to trial. Individual cells in the prefrontal cortex are selectively active during the delay period, such that some neurons fire action potentials at a high rate only when the position being remembered is at or near a specific preferred direction. This preferred direction varies from cell to cell. In this way, specific populations of neurons maintain a representation of the location of any stimulus presented in the visual field. It was later shown that sustaining this activity depends on strong connections within these networks (Compte et al., 2000). In essence, neurons with similar spatial preferences tend to excite each other, whereas neurons with dissimilar spatial preferences tend to inhibit each other, such that only a few neurons remain strongly active during the delay period.

Neurons in the prefrontal cortex are modulated by inputs from the norepinephrine system, which projects to the prefrontal cortex from the brain stem. The influence of norepinephrine is mediated via postsynaptic α 2A-ARs, and manipulation of these receptors can strongly affect work-